RESEARCH LETTER

Fetuin A and retinol-binding protein 4 are associated with insulin resistance in acromegaly

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Introduction Acromegaly is a rare disorder characterized by uncontrolled growth hormone (GH) secretion and insulin-like growth factor 1 (IGF-1) excess, which result in numerous systemic complications including cardiovascular diseases and diabetes. Uncontrolled GH secretion contributes to hyperglycemia primarily by exacerbating insulin resistance. It stimulates lipolysis, which increases the availability of free fatty acids, and consequently reduces glucose utilization in the peripheral tissues. Additionally, GH promotes gluconeogenesis, and directly inhibits insulin signaling. Although under physiological conditions IGF-1 increases glucose utilization and free fatty acid uptake by the liver / adipose tissues, in acromegaly its beneficial influence is suppressed by the detrimental effect of GH.[1] Adipose tissue dysregulation, including altered adipokine secretion, contributes to insulin resistance in acromegaly.[2] Fetuin A and retinol-binding protein 4 (RBP4), although predominantly produced in the liver, are also derived from adipose tissue.[3,4] Both these cytokines negatively affect glucose homeostasis and participate in adipose tissue inflammation and insulin resistance development.[5,6] The research on fetuin A and RBP4 in acromegaly is limited to a few publications and none of them addressed their association with glucose homeostasis.[7,8] This study aimed to assess serum fetuin A and RBP4 concentrations in patients with acromegaly, in terms of the disease activity, glucose homeostasis, lipid profile, and cardiovascular risk.

Patients and methods **Group characteristics** Initially, 64 patients with acromegaly were recruited. The individuals with acromegaly-associated diabetes (n = 13) were excluded from the study. The patients with other uncontrolled endocrine disorders, previous cardiovascular events, including stroke and myocardial infarction, chronic liver or kidney disease, and those with body mass index (BMI) above 40 kg/m^2 were not involved the study. The participants with acromegaly were divided into 2 groups, either controlled or active and biochemically uncontrolled disease, based on IGF-1 concentrations within the normal range or exceeding the upper limit, respectively. The active acromegaly group comprised 27 treatment-naïve individuals, while the controlled disease group included 24 patients (10 patients underwent pituitary surgery alone, 9 were on continuous first-generation somatostatin analogue treatment, 2 were controlled with pasireotide, 3 were treated with pegvisomant, and additionally, 5 patients received radiotherapy). Due to insufficient sample size, the groups treated with pasireotide and pegvisomant were excluded from the analyses. Supplementary material, *Figure S1* presents the recruitment process of the acromegaly patients. The control group comprised 36 healthy individuals who did not differ from the study groups in terms of age, BMI, and glycemic status.

Study protocol All the patients underwent a complete clinical examination. Blood samples were obtained after an overnight fast and analyzed for creatinine, fasting glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, and GH levels, using the Cobas 8000 modular analyzer (Roche Diagnostics, Basel, Switzerland), for IGF-1 levels using a chemiluminescence immunoassay and the Liaison analyzer (DiaSorin S.p.A., Saluggia, Italy), and for glycated hemoglobin (HbA_{1c}) using a high performance liquid chromatography D10 system (Bio-Rad Laboratories, Hercules, California, United States). Blood samples for evaluation of fetuin A and RBP4 levels were frozen at –80 °C and stored until the recruitment was completed. The measurements were performed with commercially available enzyme-linked immunosorbent assay kits of human fetuin A (BioVendor Laboratory Medicine, catalog number RD191037100, Brno, Czech Republic)

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and human RBP4 (Immundiagnostik AG, catalog number K6110, Bensheim, Germany).

Non–HDL-C level was calculated by subtracting HDL-C from total cholesterol. Homeostatic model assessment for insulin resistance (HOMA-IR) was determined using a standard formula.[9] We used a recently validated instrument, SAGIT, for comprehensive assessment of acromegaly severity.[10] The cardiovascular risk was assessed using the Systematic Coronary Risk Evaluation 2 (SCORE2) / SCORE2-older persons risk prediction algorithms.[11] The algorithms are suitable for individuals aged 40 years or older, therefore younger patients were excluded from the SCORE2 estimation and associated analyses.

Ethics The study was conducted in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the Poznan University of Medical Sciences (118/21). All participants provided their written informed consent for the study.

Statistical analysis The data are expressed as mean (SD), when normally distributed, or median (interquartile range), when non-normally distributed. Normality was determined using the Shapiro–Wilk test, while equality of variances was verified using the Levene test. For comparisons, the independent *t* test or the Mann–Whitney test or 1-way analysis of variance (ANOVA) with the post hoc Tukey test or the Kruskal–Wallis 1-way analysis of variance by ranks with the post hoc Dunn–Bonferroni test were applied. Correlations were analyzed using the Pearson product-moment correlation (r) or the Spearman rank-order correlation (R). Nominal data were compared using the Fisher exact test. A *P* value below 0.05 was considered significant. The statistical analysis was performed using the PQStat Software v.1.8.4.162 (PQStat Software, Plewiska, Poland).

Results Group characteristics are summarized in TABLE 1. Serum fetuin A concentration was significantly higher in the patients with active acromegaly than in both the controlled acromegaly group and the healthy controls. It was also significantly lower in the patients treated with the first-generation somatostatin analogues than in the healthy controls. For RBP4 levels, no differences between the groups were identified. Adipokine concentrations did not differ between women and men ($P = 0.76$ for fetuin A and $P = 0.1$ for RBP4, respectively) or between normal-weight, overweight, and obese individuals ($P = 0.29$ for fetuin A and $P = 0.79$ for RBP4, respectively), and were not influenced by smoking status ($P = 0.09$ for fetuin A and $P = 0.12$ for RBP4, respectively). We observed a tendency toward higher fetuin A and RBP4 concentrations in the patients with diabetes, as compared with those with prediabetes and normal glucose tolerance, however, the differences did not reach statistical significance.

The associations between adipokines, hormonal, and metabolic parameters in the patients with acromegaly are presented in Supplementary material, *Table S1*. Fetuin A correlated positively with IGF-1 (R = 0.689; $P < 0.001$) and SAGIT (R = 0.631; $P < 0.001$), that is, the parameters used for estimation of the disease activity, as well as with GH (R = 0.66; $P < 0.001$), while RBP4 did not correlate with these parameters. We identified no relationship between the analyzed adipokines and BMI or systolic blood pressure, though RBP4 was related to body mass (R = 0.317; $P = 0.03$). Fetuin A was negatively associated with age (r = –0.435; $P = 0.003$). In terms of glucose homeostasis, both fetuin A and RBP4 correlated positively with fasting glucose (r = 0.305; $P = 0.04$ and R = 0.361; $P = 0.01$, respectively) and HOMA-IR (R = 0.635; $P < 0.001$ and R = 0.361; $P = 0.01$, respectively), but were not related to HbA_{1c} (r = 0.111; $P = 0.46$ and R = 0.274; $P = 0.07$, respectively). In the lipid panel, we did not observe any significant associations between fetuin A, RBP4, and cholesterol fractions or triglycerides. An inverse relation between fetuin A and SCORE2 was identified (R = –0.34; $P = 0.046$).

Discussion In this study, we found that serum fetuin A concentration is increased in active acromegaly, as compared with the controlled disease group and healthy individuals, and that its concentration correlates with the disease activity assessed with biochemical and clinical measures. RBP4 levels did not differ between the active disease and healthy participants, and were not associated with IGF-1 levels or SAGIT score. We identified a relation between both of the analyzed cytokines and insulin resistance index in acromegaly patients.

Our findings are generally consistent with previous research. A single study reported no change in circulating RBP4 levels in biochemically uncontrolled acromegaly as compared to controls.[8] A research conducted in athletes, aimed at improving detection of GH abuse (doping), revealed a significant increase in circulating fetuin A levels following 8 weeks of recombinant GH administration.[12] Another study demonstrated increased fetuin A concentrations in active acromegaly, and suggested its protective role against atherosclerosis.[7] Fetuin A prevents vascular calcification that contributes to the development of atherosclerosis.[13] We observed an inverse relation between fetuin A and SCORE2, a surrogate for 10-year risk of fatal and nonfatal cardiovascular events (myocardial infarction or stroke), in the patients with acromegaly. This finding could confirm a protective role of this protein. The pathophysiology of cardiovascular complications in acromegaly is not well understood. Basic research suggests GH and IGF-1 excess may contribute to endothelial dysfunction and promote atherosclerosis, however, clinical studies investigating the markers of vascular damage showed contradictory results.[14] Whether the patients present higher degree of

TABLE 1 Characteristics and comparisons of the patients with active acromegaly, acromegaly controlled after successful neurosurgery, acromegaly controlled with the first-generation somatostatin analogue, and control group (continued on the next page)

Parameter		Active acromegaly (n = 27)	Controlled acromegaly: neurosurgery (n = 10)	Controlled acromegaly: first-generation somatostatin analogue (n = 9)	Control group (n = 36)	*P* value	Post hoc *P* value
Sex, n	M	17	0	4	17	0.004[a]	–
	W	10	10	5	19		
Active smoking, n (%)		6 (22)	4 (40)	2 (22)	14 (39)	0.47[a]	–
Age, y		45.78 (14.61)	59 (9.93)	57.89 (13.91)	48.06 (11.59)	0.01[b]	0.09[d] 0.17[e] 0.9[f] >0.99[g] 0.03[h] 0.07[i]
BMI, kg/m^2		25.66 (24.66–29.22)	26.98 (25.45–30.63)	25.61 (25.22–26.17)	25.99 (23.17–27.83)	0.6[c]	–
Overweight, n (%)		12 (44)	4 (40)	6 (67)	17 (47)	0.67[a]	–
Obesity, n (%)		5 (19)	3 (30)	1 (11)	5 (14)	0.68[a]	–
Systolic blood pressure, mm Hg		140.48 (17.76)	128.4 (11.46)	138.44 (19.38)	136.53 (18.14)	0.32[b]	–
Hypertension, n (%)		13 (48)	5 (50)	4 (44)	5 (14)	0.01[a]	–
Fasting glucose, mmol/l		5.61 (0.63)	5.33 (0.5)	5.3 (0.7)	5.34 (0.43)	0.18[b]	–
Prediabetes, n (%)		11 (41)	4 (40)	4 (44)	12 (33)	0.88[a]	–
HOMA-IR		3.61 (2.8–4.82)	2.42 (1.58–3.35)	0.98 (0.87–1.04)	2.94 (2.04–3.49)	<0.001[c]	>0.99[d] 0.001[e] 0.2[f] 0.15[g] 0.09[h] <0.001[i]
HbA_{1c}, mmol/mol		37 (34–41)	37 (37–39)	37 (37–40)	36 (32–38)	0.24[c]	–
Total cholesterol, mmol/l		4.95 (1.13)[j]	4.99 (1.09)	5.85 (1.34)	5.27 (1.03)	0.2[b]	–
LDL-C, mmol/l		2.9 (2.42–3.41)[k]	2.59 (2.13–3.26)	4.11 (2.95–4.81)	3.34 (2.6–4.05)	0.13[c]	–
HDL-C, mmol/l		1.34 (1.13–1.63)[j]	1.87 (1.53–1.96)	1.78 (1.66–2.02)	1.5 (1.16–1.79)	0.02[c]	0.46[d] 0.58[e] >0.99[f] >0.99[g] 0.05[h] 0.08[i]
Non–HDL-C, mmol/l		3.1 (2.82–4.23)[j]	2.82 (2.46–3.62)	4.63 (2.95–5.15)	3.57 (2.89–4.53)	0.35[c]	–
Triglycerides, mmol/l		1.16 (0.93–1.85)[j]	1.32 (0.99–1.65)	1 (0.84–1.26)	1.33 (1.13–2.01)	0.38[c]	–
SCORE2		7 (4–13)[l]	9 (4.5–11.75)	11 (6.25–15.75)[m]	5 (2.5–11.5)[n]	0.35[c]	–
IGF-1, nmol/l		95.6 (28.55)	25.33 (7.44)	23.98 (8.06)	22.96 (7.22)	<0.001[b]	0.98[d] >0.99[e] <0.001[f] >0.99[g] <0.001[h] <0.001[i]
Growth hormone, µg/l		9.74 (5.38–16.5)	1.05 (0.47–2.32)	1.1 (0.88–1.9)	0.3 (0.14–2.42)	<0.001[c]	>0.99[d] >0.99[e] <0.001[f] >0.99[g] 0.001[h] 0.002[i]
SAGIT score		11 (10–13)	2.5 (2–3)	2 (2–3)	–	<0.001[c]	>0.99[g] <0.001[h] <0.001[i]

TABLE 1 Characteristics and comparisons of the patients with active acromegaly, acromegaly controlled after successful neurosurgery, acromegaly controlled with the first-generation somatostatin analogue, and control group (continued from the previous page)

Parameter	Active acromegaly (n = 27)	Controlled acromegaly: neurosurgery (n = 10)	Controlled acromegaly: first-generation somatostatin analogue (n = 9)	Control group (n = 36)	*P* value	Post hoc *P* value
Fetuin A, μg/ml	286.23 (51.02)	210.49 (40.52)	201.11 (24.23)	246.29 (34.93)	<0.001[b]	0.08[d] 0.02[e] 0.001[f] 0.96[g] <0.001[h] <0.001[i]
RBP4, mg/l	25.4 (21.85–28)	26.1 (21.15–27.18)	20.6 (19.5–23.9)	25.8 (24.73–28.1)	0.26[c]	–

Data are presented as mean (SD) or median (interquartile range) unless indicated otherwise.

a Fisher exact test

b One-way analysis of variance (ANOVA) with post hoc Tukey test, when significant

c Kruskal–Wallis 1-way ANOVA by ranks with post hoc Dunn–Bonferroni test, when significant

d Control group vs controlled acromegaly: neurosurgery

e Control group vs controlled acromegaly: first-generation somatostatin analogue

f Control group vs active acromegaly

g Controlled acromegaly: neurosurgery vs controlled acromegaly: first-generation somatostatin analogue

h Controlled acromegaly: neurosurgery vs active acromegaly

i Controlled acromegaly: first-generation somatostatin analogue vs active acromegaly

j n = 22

k n = 21

l n = 17

m n = 8

n n = 27

Abbreviations: BMI, body mass index; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, Homeostatic Model Assessment – Insulin Resistance; IGF-1, insulin-like growth factor 1; LDL-C, low-density lipoprotein cholesterol; M, men; RBP4, retinol-binding protein 4; SCORE2, Systematic Coronary Risk Estimation 2; W, women

atherosclerosis and coronary heart disease due to acromegaly itself remains unclear. Further prospective studies are needed to confirm that increased levels of fetuin A, as observed in our study, may play a protective role in the pathophysiology of cardiovascular diseases in acromegaly.

We report a positive correlation between fetuin A and RBP4 levels and HOMA-IR, that is, the index reflecting insulin resistance, in acromegaly patients. This association has not been investigated yet; however, basic and clinical research works support potential contribution of fetuin A and RBP4 levels to insulin resistance development in acromegaly. Adipose tissue dysregulation is the main contributory factor of insulin resistance in acromegaly. GH impairs differentiation of adipocytes and adipogenesis, which results in reduced adipose tissue mass and its redistribution.[2] Apart from constantly enhanced GH-induced lipolysis, changes in adipokines may contribute to disordered metabolism of adipose tissue and exacerbate insulin resistance in acromegaly.[2,15] Both fetuin A and RBP4 suppress insulin secretion and signaling in target tissues, and promote adipose tissue inflammation, which result in impaired insulin sensitivity and glucose homeostasis.[5,6] A recent study identified *RBP4* as a downstream gene of the GH receptor, involved in the regulation of insulin sensitivity. Overexpression of the GH receptor enhanced circulating RBP4 levels and repressed its renal clearance.[16] This finding could further support the hypothesis that RBP4 contributes to insulin resistance in acromegaly. In contrary to previous studies in patients without acromegaly,[17,18] we did not observe significant differences in fetuin A and RBP4 levels between diabetic and normoglycemic individuals with acromegaly. Perhaps the differences would reach statistical significance when confirmed in a larger group. We could also speculate that concentrations of the analyzed cytokines are not related to the degree of hyperglycemia,

as no associations with HbA_{1c} were observed, but they rather reflect dysregulated response to insulin. We observed lower HOMA-IR and lower fetuin A concentration in acromegaly patients treated with the first-generation somatostatin analogue as compared with both the active disease group and healthy controls. A decrease in HOMA-IR is likely attributable to the inhibitory effect of this medication on insulin secretion,[19] while its potential influence on fetuin A and RBP4 levels remains unknown.

In the lipid panel, we observed no significant differences between the patients with acromegaly and the controls. A majority of the research evidence has pointed toward increased triglyceride and reduced HDL-C levels in acromegaly, with variable concentrations of LDL-C and total cholesterol.[14] RBP4 level was previously described to correlate positively with triglycerides and negatively with HDL-C, while fetuin A level was found to be associated with that of triglycerides in healthy controls.[20] Contrary to studies in nonacromegaly individuals, we did not observe significant correlations between RBP4, fetuin A, and cholesterol fractions, and triglyceride levels in our cohort. Whether the changes in adipokine levels could contribute to the lipid panel abnormalities observed in acromegaly requires further research.

We have identified several limitations of our work. This is an observational, cross-sectional study, which does not allow for drawing conclusions with respect to causality. Another issue is a relatively small sample size, which limited the applied statistical methods, including regression analyses. The sample size was also insufficient to compare the cytokine profile in the patients treated with pasireotide and pegvisomant. Additionally, the controlled acromegaly group after successful neurosurgery comprised only women, which could have influenced the study outcomes. Although a majority of research studies, including ours, demonstrated no differences in fetuin A concentration between women and men, several works reported higher fetuin A levels in women. Findings on sex-related changes in RBP4 levels are contradictory. Finally, HOMA-IR is a calculated index that cannot replace the gold standard tests assessing insulin resistance. Nonetheless, our findings offer new observations on insulin resistance in acromegaly, which should be further investigated.

In conclusion, we found that fetuin A concentration increases in active acromegaly and is related to the disease control, as assessed with clinical and biochemical measures. Additionally, we observed an association between fetuin A and RBP4 levels and the parameters of glucose homeostasis in acromegaly patients. Further prospective studies in larger groups are needed to clarify the role of adipokines in metabolic complications accompanying acromegaly and to determine the utility of their assessment in the clinical settings.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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